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Relationship between Serum Dioxin-Like Polychlorinated Biphenyls and Post-Testicular Maturation in Human Sperm

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Highlights

- Serum DL-PCB concentrations were higher in the low quality semen group.
- Individual DL-PCB congeners as 77, 81,123, 126,169,118 and 189; ∑ non-ortho and ∑ DL-PCBs could be implicated in the alterations of male fertility.
- In adult men, serum dioxin-like polychlorinated biphenyls concentrations may have adverse effects on semen quality, with an increased number of morphologically abnormal sperm, decreased motility and sperm membrane damage. However, it does not appear to deteriorate sperm concentration and total sperm count.
- Dioxin-like polychlorinated biphenyls seems to act, in the late stages of spermatogenesis (during spermiogenesis) and/or epididymal maturation, in adulthood.

Abstract

The relationship between dioxin-like polychlorinated biphenyl (DL-PCB) levels in serum and semen parameters were investigated. Our case-control included two groups of patients. Total concentrations of PCBs were significantly higher in the low semen quality (n=24) than in the normal semen quality (n=26) group.

A significant negative correlation was found between PCB 126 and viability in men with low semen quality, while PCBs 77 and 81 were positively correlated with morphology, and PCB 118, mono-ortho and total DL-PCBs were positively correlated with volume. In the normal semen quality group, PCB 189 and 118 were negatively correlated with sperm motility and volume, respectively. In addition, positive significant correlations were found between PCB 77, 23 and total non-ortho PCBs with regard to morphology. Our findings suggest that sperm motility, viability, volume and

morphology are parameters sensitive to alteration by exposure to DL-PCBs, although PCB effects on spermatogenesis were not of clinical significance.

KEY WORDS: male fertility, human serum, DL-PCBs, semen parameters, endocrine disruptors.

1. Introduction

Infertility is an important health problem in both the developed and developing worlds, with up to one in six couples requiring specialist investigation or treatments in order to conceive [1]. In this context, approximately 20-50% of infertility cases are linked to male fertility factors. In 30-40% of cases, no male-infertility-associated factor is found (idiopathic male infertility), with normal parameters in endocrine, genetic and biochemical laboratory testing [2]. However, when semen analyses are performed, a spermiogram reveals several sperm pathological parameters affecting fertility [3]. It has been hypothesised that environmental exposure to toxic pollutants and endocrine disruptors such as pesticides, phthalates, polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) or specifically polychlorinated biphenyls (PCBs) plays an important role in these trends [4].

PCBs are persistent and lipophilic organochlorines, formerly used in cutting oils and lubricants and as electrical insulators. PCB use and manufacture was discontinued in many countries in the late 1970s in view of their toxicological effects on humans and laboratory animals [5]. Today, substantial exposure to PCBs persists through the ingestion of food, mainly fish, meat and dairy products [6].

Moreover, some of the PCB congeners (non-ortho and mono-ortho PCBs) exhibit similar biological toxicity to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a

congener of dioxins considered the most highly toxic environmental contaminant ever manufactured [7], and are therefore often termed “dioxin-like PCBs” (DL-PCBs). These compounds possess oestrogenic and anti-oestrogenic properties [8] and act through the cytosolic aryl hydrocarbon receptor / aryl hydrocarbon receptor nuclear translocator (AhR/ARNT) receptor complex, which is often called “the dioxin receptor” for this reason [9].

The male reproductive system is highly sensitive to exposure to these compounds [10]. Over the past 20 years several chemicals have appeared in our environment, and these may interfere with endogenous hormone signalling or act as hormones themselves, leading to steroid hormone receptor interferences [11]. Studies in animals have reported adverse effects involving dioxins or dioxin-like chemicals following both developmental exposures and exposure in adulthood, including reproductive defects and steroid hormone function disruption [12, 13, 14].

However, few epidemiological studies have investigated the relationship between male reproductive function and DL-PCB exposure [15]. For example, Guo et al. showed that accidentally high prenatal exposure to PCBs/PCDFs induced alterations in sperm morphology, decreased sperm motility, and reduced oocyte penetration capacity in exposed men [16]. Dhooge et al. [17] observed in young men that semen volume decreased but sperm concentration increased with high levels in dioxin-like activity (using the “Chemical Activated LUciferase gene eXpression” or CALUX assay). In addition, in the large population of Russian boys aged 8-9 to 17-18, associations between exposures to DL-PCBs and delayed puberty were found [18].

However, the effects of exposure at older ages are less noticeable, and consequently there is little epidemiologic evidence on the effects of DL-PCBs on male reproduction in humans [17]. In a multinational study (Poland, Greenland, Ukraine and

Sweden) on adult men, Toft et al. found no strong and consistent associations between environmental exposure to dioxin-like compounds and semen quality measurements using CALUX [19]. In contrast, another study found positive relationships between sperm quality measurements and PCBs (dioxin-like PCB-118, and non-dioxin-like PCBs 138, 153 and 180) in men with poor semen quality and in controls, whereas sperm progressive motility and concentration were inversely related to the levels of PCB metabolites [20]. Cross-sectional studies of men who were partners in subfertile couples have found inverse associations between serum concentrations of PCB 153, 138, 118, ∑PCBs, and PCBs classified as enzyme inducers, and semen quality, particularly sperm motility [21, 22].

The aforementioned studies focus on the relationship between only one individual DL-PCB (PCB-118), whereas the rest are non DL-PCBs and/or congener groups, with seminal parameters in subfertile men. As a consequence, there is a need to analyse individual congeners of DL-PCBs and their potential effects on the reproduction system of adult men. Hence, the aim of the present study was to investigate whether the concentration of DL-PCBs in serum could be correlated with semen quality in subfertile males.

2. Materials and Methods

2.1. Design and patients

This investigation was designed as an analytical observational case-controlled study. The objects of the study were adult males (n = 56) (30-55 age range) who were partners in subfertile couples seeking infertility diagnosis from the IVF Spain clinic (Alicante, Spain) during the period from May 2012 to June 2014. Consecutive eligible individuals (from couples not affected by infertility factors) were selected to participate.

2.1.1. Questionnaire

A complete clinical examination was performed on every patient, and a questionnaire was used to collect individual information, including personal background, lifestyle factors, medical history, tobacco/alcohol consumption and likely exposure to environmental chemical compounds. Informed consent was given by every patient after receiving a detailed explanation of the study. Participation of human subjects did not occur until written informed consent was obtained. The ethics committee of the University General Hospital of Alicante approved the study in accordance with the principles of the Declaration of Helsinki.

2.1.2. Exclusion criteria

Patients with known factors related to male infertility such as varicocele, post-vasectomy or cryptorchidism, endocrine hypogonadism (abnormal hormonal concentrations), immune infertility, genetic disease, infection, anomalies in the karyotype or Y chromosome microdeletions were excluded from the study (6 out of 56 subjects, resulting in a final $n = 50$).

Finally, the patients were divided into two groups on the basis of semen quality according to the World Health Organization (WHO) criteria [23] as follows: (i) low semen quality ($n = 24$), composed of patients with alteration of at least one parameter in the semen analysis and, (ii) a control group ($n = 26$), composed of patients with normal semen quality, with all the semen parameters above the WHO 2010 cutoffs.

2.3. Semen analysis

Semen samples were collected by masturbation into sterile cups following 3 days of sexual abstinence. After 30 minutes of liquefaction at 37 ºC, standard semen parameters (sperm concentration, volume, percent motile sperm, and percent morphologically normal sperm) were immediately evaluated according to WHO criteria

[23]. Ejaculate volumes were estimated by weighing the sample in the vessel in which it was collected. In order to measure sperm concentration and motility, we placed aliquots of semen samples (5 µL) into a prewarmed (37 ºC) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel); a minimum of 200 sperm from at least four different fields were analysed from each individual sampled. Sperm motility was classified into four categories as follows: rapid progressive motile (Type a), slow progressive motile (Type b), non-progressive motile, and immotile spermatozoa. In addition, sperm morphology was measured on air-dried Papanicolaou-stained seminal smears by recording the percentage of normal forms following Kruger's strict criteria [24]. Finally, the total sperm count was derived by multiplying the individual's sperm concentration and volume [23]. Semen analyses were performed by the same technician, who was blind to other clinical data.

2.4. Measurement of serum DL-PCBs

Blood serum and semen samples were collected from each subject on the same day. Target analytes included 12 DL-PCBs from non-ortho PCBs (77, 81, 126 and 169) and mono-ortho PCBs (105, 114, 118, 123, 156, 157, 167 and 189). The blood samples were obtained by venipuncture and collected in Vacutainer test tubes without anticoagulant, and were immediately transferred to a glass centrifuge tube. After centrifugation and decantation the serums were stored in the dark at -20ºC until they were analysed.

Analytical methods and quality control were carried out according to Moltó et al. [25]. Briefly, serum samples were spiked with $^{13}C_{12}$-labelled internal standards before extraction (WP-LCS; Wellington Laboratories Inc., Guelph, Canada), extracted several times using hexane, and purified using sulphuric acid. All of the reagents were for the organic trace analysis and were purchased from Merck (Germany). Finally, the extracts

were analysed by means of high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS) using the isotopic dilution method in an Agilent HP5890 gas chromatograph equipped with a programmable temperature vaporisation (PTV) injector, coupled to a Micromass Autospec Ultima-NT mass spectrometer, using an Agilent DB5-MS chromatographic column (60 m × 0.25 mm i.d. × 0.25 μm).

PCB concentrations were adjusted for total serum lipids. DL-PCB concentrations are reported both individually and as the sum of all congeners assayed (total DL-PCBs) and expressed in picograms of compound per gram of total lipids (pg/g lipid). Serum total cholesterol and triglycerides were measured enzymatically, and total lipid concentrations calculated by the Phillips formula [26].

The toxic equivalent units (TEQ) were then calculated based on the serum concentration multiplied by the respective toxic equivalent factor (TEF) according to WHO-TEFs-2005 [27]. We also expressed the total TEQs (total WHO-TEQ DL-PCBs) as the sum of the TEQs obtained from the DL-PCBs measured in the serum samples.

Quality assurance criteria were based on the minimum requirements described in US EPA method 1668C for dioxin-like PCBs [28]. A procedural blank was associated with each batch of 4 samples and processed in the same manner.

2.5. Statistical analyses

The non-parametric Mann-Whitney *U*-test was used to assess differences between low- and normal-quality semen parameters. Multivariate linear regressions were used to evaluate the relationship between DL-PCB levels and semen parameters for both low- and normal-quality semen participants. Multivariate regression models were created with continuous semen parameters as dependent variables and DL-PCBs as predictor or independent variables [29, 30] and considering the DL-PCBs from non-

ortho PCBs (77, 81, 126 and 169) and mono-ortho PCBs (105, 114, 118, 123, 156, 157, 167 and 189). In the present study, congeners below the limit of detection (LOD) were reported considering a concentration equal to their respective LOD. For each of the 12 DL-PCBs measured, the percentage of samples with levels above the LOD was calculated. Those analytes with at least 60% of the values above the LOD were included in further analysis (in our study, all congeners), following early reports [31, 32]. Moreover, the following semen parameters (dependent variables) were included in the model: semen volume (mL), sperm concentration ($x10^6$/mL), sperm total count ($x10^6$), sperm progressive motility (%), sperm viability (%) and normal morphology (%) following Kruger's criteria [24], because of relevance in the relationship between male reproductive function and PCB concentrations [21, 29, 30]. We considered that a correlation was present when we found a statistically significant linear trend between PCBs and the semen parameters selected. A *p*-value of <0.05 was considered to be statistically significant. Descriptive and statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of participants in the study

The characteristics of the population are described in Table 1. Participants were men of high and medium socioeconomic status without specific occupational exposure to DL-PCBs. The overall mean age and body mass index (BMI) were 38.04 ± 5.01 years and 24.77 ± 2.28 kg/m^2, respectively (expressed as mean ± standard deviation (SD)). All of them were white, 84% had never smoked and 6% had never consumed alcohol. Non-significant differences were found in age, BMI, smoking status, alcohol and drug intake, level of education, employment and socioeconomic status, and residential area,

when normal and low-quality semen parameters were compared ($p < 0.05$). The sexual abstinence time was approximately 3 days; since the study design controlled for sexual abstinence time and this variable was not significant, there was no need to control for it in the analysis.

Seminal parameter results are shown in Table 2. We found that individuals with low semen quality showed significant impairment of sperm concentration (24.70 ± 33.97 $x10^6$/mL), total sperm count (38.05 ± 52.84 $x10^6$), motility (20.22 ± 23.55 %), viability (62.28 ± 21.93 %) and morphology (5.36 ± 4.85 %) ($p < 0.001$). However, semen volume, cholesterol and triglycerides showed similar values for both low- and normal-quality semen groups.

3.2. Concentrations of PCBs

Concentrations of PCBs expressed as pg-WHO-TEQ/g lipid in the serum samples are summarised in Figure 1A. The mean value of the total DL-PCBs (22.52 ± 21.2 pg WHO-TEQ/g lipid) and the non-ortho PCBs (22.32 ± 21.33 pg WHO-TEQ/g lipid) in the group of patients with low quality semen were higher than in the normal quality semen group (14.00 ± 10.82 pg WHO-TEQ/g lipid and 13.85 ± 10.69 pg WHO-TEQ/g lipid, respectively), but the differences were not statistically significant. However, mono-ortho PCBs in low semen quality patients (0.2 ± 0.13 pg WHO-TEQ/g lipid) were statistically higher ($p < 0.05$) than in the control group (0.15 ± 0.13 pg WHO-TEQ/g lipid).

In addition, the mean values of the DL-PCB concentrations (expressed as pg/g lipid) in serum of the males studied were also compared between both groups (see Figure 1B). We observed that individuals with altered semen parameters exhibited significantly higher levels of non-ortho PCBs (949.49 ± 624.97 pg/g lipid; $p = 0.020$) and total DL-PCBs (7029.96 ± 3023.97 pg/g lipid; $p = 0.028$) than in the control group

(508.40 ± 324.44 pg/g lipid and 4805.92 ± 2205.02 pg/g lipid, respectively). Furthermore, mono-ortho PCBs of subfertile patients (6080.46 ± 2754.89 pg/g lipid) and controls (4297.52 ± 2030.13 pg/g lipid) also tend to show significant differences ($p < 0.05$).

The concentrations of each congener are listed in Table 3 in terms of mean ± standard deviations (SD). Results were expressed both as pg/g lipid and in terms of toxicity (pg WHO-TEQ/g lipid). The respective limits of detection (LOD) and *p*-values between groups can also be found in Table 3. When the concentrations of each PCB were compared between the two groups, we only found statistical significance for PCB 105 ($p = 0.031$). Moreover, the most toxic of the DL-PCBs, PCB-126, showed a higher value in the low semen quality group (15.23 ± 13.83 pg WHO-TEQ/g lipid) compared with normal semen quality group (9.96 ± 8.14 pg WHO-TEQ/g lipid).

PCB congeners 118, 156 and 105 were the most abundant, with mean values in the low semen quality and normal semen quality groups of 2695.31 vs. 1877.31 pg/g lipid, 1189.29 vs. 874.68 pg/g lipid and 821.13 vs. 451.38 pg/g lipid, respectively.

3.3. Relationship between serum DL-PCBs and sperm parameters

For the low semen quality group, the data showed a negative significant correlation between PCB 126 in serum, the most toxic dioxin-like PCB, and viability ($r = -0.645$; $p = 0.013$). Moreover, sperm morphology was positively correlated with two non-ortho PCBs, PCB 77 ($r = 0.671$; $p = 0.009$) and PCB 81 ($r = 0.552$; $p = 0.041$). Finally, positive correlations between sperm volume and PCB 118 ($r = 0.556$; $p = 0.039$), total mono ortho PCBs ($r = 0.583$; $p = 0.029$) and total DL-PCBs ($r = 0.593$; $p = 0.025$) were found (Table 4).

Moreover, within the normal semen quality group, corresponding positive correlations were significant between sperm morphology and PCB 77 (r = 0.553; p = 0.026), PCB 123 (r = 0.559; p = 0.024) and, total non-ortho PCBs (r = 0.548; p = 0.028). Other parameters, such as semen volume and sperm motility, showed a statistically significant negative correlation with PCB 118 (r = -0.539; p = 0.031) and PCB 189 (r = -0.521; p = 0.039), respectively (Table 5).

When the data from the overall group (n=50) were analysed (Table 6), a statistically significant negative correlation of sperm progress motility (%) with PCB 126 (r =-0.381; p = 0.037) and PCB 189 (r = -0.410; p = 0.024) was found. We also found a highly significant relationship between sperm viability with PCB 126 (r = -0.557; p = 0.001), PCB 169 (r = -0.542; p = 0.002) and PCB 189 (r = -0.580; p < 0.001). In addition, a significant negative relationship between viability and non-ortho PCBs (r = -0.505; p = 0.004) and the total levels of DL-PCBs (r = -0.412; p = 0.023) was found. Moreover, sperm morphology was positively correlated with PCB 123 (r = 0.373; p = 0.042) but negatively correlated with PCB 189 (r = -0.440; p = 0.014). Other semen parameters including semen concentration, seminal volume and total sperm count show a lack of correlation with neither PCB concentrations.

4. Discussion

The present study shows tentative results on the relationship between DL-PCB concentrations in serum and semen quality from subfertile men. Our findings show that the total concentrations of PCBs were significantly higher among individuals with low semen quality than in normal semen quality counterparts. The WHO-TEQ values of total PCBs and non-ortho PCBs were higher, although not significantly different, in individuals with low semen quality. Likewise, there was a significantly higher level of

mono-ortho PCBs in the low-quality semen group. Thus, these data show trends that are suggestive of a relationship between DL-PCBs and the sperm parameters evaluated.

In comparison with early reports, we found that the total DL-PCBs value in the low semen quality group was similar (22.52 pg WHO-TEQ/g lipid) to those reported previously in Spanish populations (20.47 pg WHO-TEQ/g lipid) [33] and in the Scarlino area in Italy (21.2 pg WHO-TEQ/g lipid) [34]. However, the values obtained in our study were higher than the median DL-PCB concentrations reported in the serum from populations in Greece [35] and Australia [36].

The differences in DL-PCB levels found among populations may be attributed to variations in dietary intake. In fact, the consumption of fish is likely to be the most relevant source of PCBs for humans [37]. In our study, the participants were from northern Europe, where fatty fish consumption is high [38]. In fact, Swedish and Finnish estimations reveal that 70–80% of the dioxin-like PCB intake originates from consumption of fatty fish [39].

We used multivariate linear regression models to assess the relationship between the concentration of each PCB in serum with typical sperm parameters such as motility, morphology, volume and viability with a view to exploring the adverse effects of PCBs on male fertility. For the entire group, a significantly negative correlation between sperm progressive motility with non-ortho PCB 126 and mono-ortho PCB 189 was finally observed. Further, viability was negatively correlated with non-ortho PCBs (126 and 169), mono-ortho PCB 189 and the total PCB level. In addition, sperm morphology was positively correlated with PCB 123, though negatively associated with PCB 189.

We also carried out the analysis to establish the relationship between each PCB and semen quality for each group of patients. In the low semen quality subgroup, we found a strong negative correlation between non-ortho PCB 126 levels in serum and

viability, while sperm morphology correlated positively with other individual congeners (non ortho PCBs 77 and 81). In the group of men with normal semen quality we found a statistically significant negative correlation between sperm progressive motility and mono-ortho PCB 189. Moreover, sperm morphology correlated positively with PCBs 77 and 123. In this context, previous reports found a reduced motility and increased abnormal morphology relationship with PCB/dioxin exposure [16, 40]. However, the positive correlation found between the percentages of morphologically normal sperm with some specific congeners is contrary to the results on the effects of PCBs on sperm morphology previously reported [41]. The resulting variation may be due to structural differences between PCBs. The rate and extent of metabolism within PCBs depend on the number and positions of the chlorine atoms in the molecule [42]. Thus, differences based on the metabolism of each congener could express a variety of toxic effects on sperm cell biomarkers.

In the present study, semen volume and its relationship with each PCB was also investigated. We found an inverse correlation with PCB 118, which reached negative significance only in those subjects with good sperm parameters. A similar relationship was found by Dhooge et al. [17] in men from the general population. In contrast, within the group of subjects with low semen quality, we found a positive significant relationship with this same congener. However, for the whole group no relationship was found. The effects of xenoestrogens on the endocrine system and their impact on reproductive health are multiple. For instance, these toxins or their metabolites may act directly on accessory glands by altering the quality or quantity of their secretions and this could influence semen volume [43]. Further studies on the effect of DL-PCB exposure –both individual congeners and PCB mixtures– on male accessory glands are required.

Interestingly, the lack of correlation between serum PCB levels and both the total sperm count and sperm concentration (for the entire group as well as the individual groups studied) was similar to those obtained in other mammals like adult male rats, in which exposure to TCDD at relatively high doses caused a decrease in weight of two androgen-sensitive organs (seminal vesicles and epididymis) without affecting spermatogenesis [44]. Similarly, studies performed with human adult males suggest no relationship between dioxin-like activity and sperm concentration [19]. By contrast, other authors have reported relationships between human prenatal and postnatal exposure to dioxin compounds and alterations in semen quality [45]. Previously, another study showed that exposure to low levels of these compounds before puberty induces a reduction in sperm concentration; however, no effects were observed during adulthood [46]. In a recently published study, Burns et al. analysed the association of peripubertal serum levels of dioxin-like compounds and non-dioxin-like PCBs with pubertal onset and maturity among Russian boys, who were enrolled at ages 8-9 and followed prospectively through ages 17-18 [18]. The authors found robust associations of serum levels of dioxin-like compounds with later pubertal onset and sexual maturity, while non dioxin-like PCBs were associated with earlier pubertal onset and sexual maturity. In another recent study, higher peripubertal serum TCDD concentrations and PCDD TEQs were associated with poorer semen parameters in young Russian men [47].These findings suggest that the developing male reproductive tract is particularly sensitive to exposure to environmental chemicals.

In the light of those results, the level of exposure, the age at the time of exposure (before and after puberty), the duration of such exposure and other endogenous or environmental factors may play an important role on the observed effects. Furthermore, this could explain, in part, the lack of effect of these chemicals on spermatogenesis of

the mature reproductive system [46]. The impact of PCB exposure on semen quality appears to affect differentiation of spermatids (spermiogenesis) and epididymal maturation, which in turn would manifest as decreased sperm motility and morphology [48,49]. Epididymal and accessory gland functions are strongly regulated by sex hormones and express AhR, androgen receptors (AR) as well as oestrogen receptors α and β (ERα, ERβ) [50] as potential targets of toxic action affecting sperm motility and sperm DNA integrity [51].

Overall, several causes may contribute to the relationships observed in this study between DL-PCB exposure and altered semen quality. As already mentioned, TCDD and other dioxin-like chemicals produce dramatic effects primarily through the aryl hydrocarbon receptor (AhR) according to Sorg [9]. AhR and ARNT are expressed in all seminiferous tubule stages of the human testes and in spermatocytes [52, 53], where they are thought to play an important role in normal sperm development [54]. The effect of AhR signalling could be stimulatory or inhibitory, depending on other factors such as the level of dioxin exposure, the period of sensitivity and/or development of the target cells, and the actual level of key regulatory molecules, including the androgen-oestrogen balance [46]. Moreover, polymorphisms in genes involved in AhR signalling have also been associated with impaired semen quality [55].

Alternatively, it is well established that dioxin compounds may produce oxidative stress in the male reproductive tract [56]. An increase in oxidative stress can be seen in approx. 80 % of clinically proven infertile men, since exposure to environmental toxicants is a major factor contributing to such an increase [57]. Human spermatozoa are particularly susceptible to oxidative stress owing to their high polyunsaturated fatty acid content. Although small amounts of reactive oxygen species (ROS) are required for normal sperm functioning, their excess production can cause

lipid peroxidation with plasma membrane and mid-piece defects affecting sperm motility [58]. ROS can also induce oxidation of critical sulfa-hydroxyl (SH) groups in proteins and DNA, with direct implications for spermatogenesis and cellular functionality [59].

The present study has a number of limitations. First, the number of participants included in the study was small. As a consequence, the results of these analyses must be interpreted with caution. In addition, because the study was conducted among men recruited through a fertility clinic, the findings of our study cannot be generalised. For a better understanding of the potential effects of environmental chemicals on semen quality, studies involving wider populations would be needed to confirm these preliminary findings. However, these results may still be useful to men seeking fertility care.

The strengths of our study include the fact that we measured and reported not only total PCBs and TEQs, but also 12 individual congeners with a similar mode of action. The detailed congener-specific PCB analysis provides a more comprehensive and accurate population (or subgroups) pattern of DL-PCB exposure and represents a valuable tool to assist in the determination of exposure pathways for individuals. For example, highly chlorinated congeners such as PCBs 105, 118 and 156 were related with occupational exposure [60, 61]. In addition, lower chlorinated congeners may indicate indoor air exposure [62], while levels of non-dioxin like PCBs 153 and 180 might reflect general (dietary) exposures [63]. Unfortunately, we did not measure non-dioxin-like PCBs. Further, more detailed studies are needed to investigate dioxin-like and other PCBs – both individual congeners and PCB mixtures – in male fertility.

The congener profile of DL-PCBs in serum samples could be of help in designing appropriate measures to ensure a safer and healthier work environment and/or

changes in lifestyle in order to reduce exposure to PCBs, which might improve the fertile capacity in infertile couples. Furthermore, construction of the toxicological profile of PCBs reveals the presence of several congeners that have the potential to affect semen quality and allows a better understanding of the toxicological/epidemiological consequences of exposure to hazardous substances on male reproductive health.

5. Conclusions

In summary, our findings suggest that the negative effects of dioxins occur in the late stages of spermatogenesis (during spermiogenesis) and/or epididymal maturation, resulting in the alteration of certain semen parameters such as motility, volume, morphology and viability. Clinicians should consider evaluating DL-PCB congener profiles in the comprehensive study of male partners from couples with unexplained infertility even in those subjects with normal semen quality. Further studies are necessary in order to better understand the effects of environmental toxics on male reproductive health.

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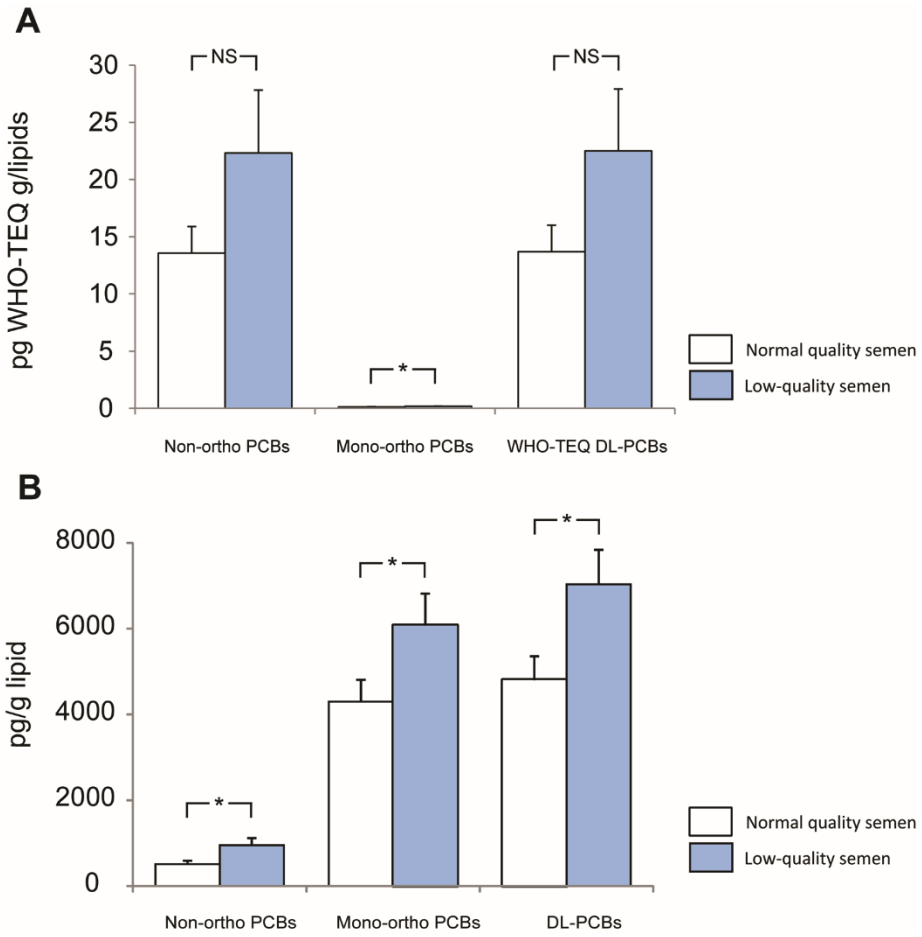

Figure 1. Comparison of the levels in serum of non-ortho, mono-ortho and Total DL-PCB concentrations expressed as pg WHO-TEQ/g lipid (A) and pg/g lipid (B) between normal and low quality semen groups. Mann-Whitney U-test at $p<0.05$ (*); not significant (NS).Error bars denote ± 1 s.e.m.

Table and Figure Captions

Table 1. Demographic characteristics of participants in the study.

Characteristics	**Total (*n* = 50)**	**Low semen quality (*n* = 24)**	**Normal semen quality (*n* = 26)**
Age (years) [Mean ± SD]	38.04±5.01	43.5±5.63	39.5±6.01
BMI (kg/m^2) [Mean ± SD]	24.77 ± 2.28	24.45±1.79	25.1±1.59
BMI (kg/m^2) [*n* (%)]			
<25	38 (76%)	19 (79.2%)	19 (73%)
25-30	12 (24.01%)	5 (20.8%)	7 (26.8%)
Smoking status [*n* (%)]			
Never smoker	42 (84%)	20 (83.3%)	22 (84.6%)
Ex-smoker	5 (10%)	2 (8.3%)	3 (11.5%)
Current smoker	3 (6%)	2 (8.3%)	1 (3.8%)
Drinking status [*n* (%)]			
Never drinker	3 (6%)	1 (4.2%)	2 (7. 7%)
Ex-drinker	42 (84%)	20 (83.3%)	22 (84.6%)
Current drinker	5 (10%)	3 (12.5%)	2 (7.7%)
Drug intake [*n* (%)]			
Never	37 (74.2%)	17 (70.8%)	20 (76.9%)
Ever*	13 (25.7%)	7 (29. 2%)	6 (23%)
*Last 3 months	10 (20.8%)	6 (25 %)	4 (15.3%)
Level of studies			
Primary education	6 (12%)	2 (8%)	4 (15%)
High school	13 (26%)	6 (25%)	7 (26%)
University	31 (62%)	16 (67%)	15 (58%)
Employment status [*n* (%)]			
Lawyer, School, IT	42 (84%)	19 (79.2%)	23 (88.5%)
Clerks	8 (16%)	5 (20.8%)	3 (11.5%)
Socioecomic status [*n* (%)]			
High	18 (36%)	9 (37.5%)	9 (34.6%)
Middle	30 (60%)	14 (58.3%)	16 (61.5%)
Low	2 (4%)	1(4.2%)	1 (3.8%)
Residential area [*n* (%)]			
Urban	27 (54%)	13 (54.1%)	14 (53.8%)
Rural	23 (46%)	11 (45.8%)	12 (46.1%)

SD: standard deviation.
n: number of patients.
*****A total of 10% of the participants reported to be on drugs occasionally during the last three months, mainly marijuana; no other kinds of drugs were reported.

Table 2. Descriptive statistics for semen parameters between low and normal quality semen participants.

Parameter[a]	**Low semen quality (*n* = 24) Mean ± SD**	**Normal semen quality (*n* = 26) Mean ± SD**	***p***[b]
Semen volume (mL)	2.48 ± 1.55	2.82 ± 0.91	0.472
Sperm concentration (x10^6 /mL)	24.70 ± 33.97	73.25 ± 52.99	0.000*
Sperm total count (x10^6)	38.05 ± 52.84	184.94 ± 116.33	0.000*
Sperm progressive motility (%)	20.22 ± 23.55	59.44 ± 13.62	0.000*
Sperm viability (%)	62.28 ± 21.93	86.81 ± 6.79	0.000*
Normal morphology (%)	5.36 ± 4.85	13.50 ± 4.75	0.000*
Cholesterol (mg/dL)	195.07 ± 22.96	192.75 ± 36.31	0.273
Triglycerides (mg/dL)	133.78 ± 52.07	130.25 ± 55.22	0.972

SD: standard deviation.
[a]Values indicate *n* (%)
[b]Mann-Whitney *U*-test at $p<0.001$ (*)

Table 3. Concentrations of DL-PCBs in serum (mean ± SD) in low and normal quality semen groups.

Isomers	Low semen quality (*n* = 24)	Normal semen quality (*n* = 26)	WHO-TEF (2005)[a]	Low semen quality (*n* = 24)	Normal semen quality (*n* = 26)	LOD	% > LOD	*p*[b]
	pg/g lipid			pg WHO-TEQ/g lipid		pg		
no-ortho PCBs								
PCB-77	492.61 ± 519.96	222.51 ± 331.34	0.0001	0.05 ± 0.05	0.02 ± 0.03	2.9	75	0.109
PCB-81	71.22 ± 42.09	57.49 ± 24.07	0.0003	0.02 ± 0.01	0.02 ± 0.01	3.2	70	0.294
PCB-126	152.47 ± 138.27	99.56 ± 81.37	0.1	15.23 ± 13.83	9.95± 8.14	0.25	65	0.224
PCB-169	233.18 ± 243.62	128.84 ± 83.48	0.03	6.99 ± 7.31	3.86 ± 2.51	0.24	83	0.147
mono-ortho PCBs								
PCB-105	821.13 ± 506.29	451.38 ± 322.87	0.00003	0.03 ± 0.02	0.01 ± 0.01	0.24	100	0.031*
PCB-114	189.12 ± 171.21	121.87 ± 108.36	0.00003	0.01 ± 0.01	0.01± 0.01	0.25	90	0.220
PCB-118	2695.31 ± 1337.61	1877.31 ± 1135.20	0.00003	0.08 ± 0.04	0.06 ± 0.03	0.25	100	0.085
PCB-123	192.28 ± 177.53	216.28 ± 419.79	0.00003	0.01 ± 0.01	0.01 ± 0.01	0.61	85	0.837
PCB-156	1189.29 ± 786.62	874.68± 413.15	0.00003	0.04 ± 0.02	0.03 ± 0.01	0.60	100	0.195
PCB-157	264.60 ± 163.69	228.23 ± 200.79	0.00003	0.01 ± 0.01	0.01 ± 0.01	0.65	68	0.589
PCB-167	294.54 ± 261.99	275.31 ± 146.27	0.00003	0.01 ± 0.01	0.01 ± 0.01	0.65	94	0.810
PCB-189	434.22 ± 392.76	252.46 ± 126.56	0.00003	0.01 ± 0.01	0.01 ± 0.04	0.21	100	0.118
DL-PCBs	7029.96±3023.97	4805.92±2205.02		22.52±21.2	14.00±10.82			

SD: Standard deviation.

[a]TEF: Toxic equivalency factors [30]

[b]Mann-Whitney *U*-test at $p<0.05$ (*)

Table 4. Multivariate regression between semen parameters and levels of DL-PCBs for low quality semen participants.

	Semen volume (mL)				**Sperm concentration (x10^6 /mL)**				**Sperm total count (x10^6)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	31.172	414.240	0.096	0.743	-1.624	532.720	-0.106	0.718	-0.158	498.630	-0.016	0.957
PCB-81	4.932	58.828	0.188	0.520	-0.035	72.103	-0.029	0.923	-0.057	73.396	-0.071	0.808
PCB-126	41.084	49.176	0.477	0.085	-1.683	194.040	-0.413	0.142	-0.753	181.130	-0.288	0.318
PCB-169	39.808	133.090	0.262	0.365	-1.489	269.940	-0.208	0.477	-1.109	275.380	-0.241	0.408
PCB-105	122.320	513.590	0.388	0.171	-1.531	858.960	-0.103	0.727	-0.503	840.260	-0.052	0.859
PCB-114	34.743	101.770	0.326	0.256	-1.175	218.150	-0.233	0.422	-0.234	198.040	-0.072	0.806
PCB-118	462.860	1531.500	0.556	0.039*	1.579	2656.300	0.040	0.892	3.453	2563.900	0.136	0.642
PCB-123	42.606	85.158	0.385	0.174	-1.293	224.210	-0.247	0.394	-0.478	210.470	-0.142	0.627
PCB-156	155.580	798.130	0.318	0.269	-2.376	1248.000	-0.103	0.727	-3.750	1332.000	-0.252	0.385
PCB-157	31.091	186.430	0.305	0.289	0.344	256.110	0.071	0.809	0.500	245.570	0.161	0.581
PCB-167	75.936	103.610	0.465	0.094	-2.101	346.430	-0.272	0.346	-2.045	372.360	-0.412	0.143
PCB-189	74.790	246.180	0.306	0.288	-1.994	483.490	-0.172	0.555	-1.421	488.300	-0.191	0.513
non-ortho PCBs	117.000	655.340	0.301	0.296	-4.830	1068.800	-0.263	0.365	-2.077	1028.500	-0.176	0.548
mono-ortho PCBs	999.930	3566.400	0.583	0.029*	-8.548	6291.600	-0.105	0.720	-4.479	6250.900	-0.086	0.770
∑DL-PCBs	1116.900	4221.700	0.593	0.025*	-13.378	7360.400	-0.150	0.608	-6.556	7279.400	-0.115	0.697
	Sperm progressive motility (%)				**Sperm viability (%)**				**Normal morphology (%)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	0.416	484.210	0.019	0.949	-3.309	698.690	-0.140	0.634	71.972	107.050	0.671	0.009*
PCB-81	0.227	66.644	0.127	0.666	0.450	43.177	0.235	0.419	4.794	45.550	0.552	0.041*
PCB-126	-2.081	194.550	-0.354	0.214	-4.065	405.640	-0.645	0.013*	-4.165	174.780	-0.146	0.619
PCB-169	-2.748	288.740	-0.266	0.359	-5.891	600.120	-0.530	0.051	-15.955	318.650	-0.317	0.269
PCB-105	-1.795	857.430	-0.083	0.777	-3.680	1050.300	-0.159	0.586	45.521	577.270	0.436	0.119
PCB-114	-1.717	223.840	-0.236	0.416	-2.165	323.980	-0.277	0.337	1.526	180.950	0.043	0.883
PCB-118	-2.860	2753.100	-0.050	0.864	-17.566	3789.400	-0.288	0.318	-43.631	2929.000	-0.158	0.589
PCB-123	-1.843	229.550	-0.244	0.400	-1.011	255.260	-0.125	0.670	12.638	124.580	0.345	0.227
PCB-156	-4.379	1277.800	-0.131	0.655	-1.487	1281.900	-0.041	0.888	-33.362	1368.000	-0.206	0.481
PCB-157	1.369	236.920	0.197	0.500	-1.557	361.600	-0.209	0.474	3.884	243.790	0.115	0.696
PCB-167	-3.538	366.070	-0.318	0.268	-0.605	332.220	-0.051	0.863	-12.446	361.210	-0.230	0.429
PCB-189	-3.927	513.630	-0.235	0.418	-9.422	1021.100	-0.526	0.053	-33.824	615.420	-0.417	0.138
non-ortho PCBs	-4.186	1034.100	-0.158	0.590	-12.814	1747.600	-0.450	0.107	56.646	646.040	0.439	0.116
mono-ortho PCBs	-18.689	6458.400	-0.160	0.585	-37.494	8415.800	-0.298	0.300	-59.694	6400.300	-0.105	0.721
∑DL-PCBs	-22.876	7492.500	-0.178	0.542	-50.308	10163.000	-0.365	0.200	-3.047	7046.300	-0.005	0.987

β_1 indicate regression slope and β_0 the intercept. Significant correlation (*r* Pearson) at $p<0.05$ (*)

Table 5. Multivariate regression between semen parameters and levels of DL-PCBs for normal quality semen participants.

	Semen volume (mL)				**Sperm concentration (x10^6 /mL)**				**Sperm total count (x10^6)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	-24.646	292.910	-0.073	0.789	-0.444	255.010	-0.071	0.794	-0.176	255.030	-0.062	0.820
PCB-81	-1.109	60.657	-0.045	0.869	0.031	55.254	0.067	0.805	0.013	55.078	0.063	0.817
PCB-126	-2.249	105.980	-0.027	0.921	0.392	70.837	0.255	0.340	0.266	50.380	0.380	0.146
PCB-169	-35.679	230.750	-0.418	0.107	0.078	123.130	0.050	0.855	-0.063	140.450	-0.087	0.747
PCB-105	-115.070	780.040	-0.348	0.186	0.247	433.270	0.041	0.881	0.060	440.320	0.022	0.937
PCB-114	-21.822	184.200	-0.197	0.465	-0.265	141.310	-0.130	0.632	-0.151	149.770	-0.162	0.549
PCB-118	-626.020	3665.400	-0.539	0.031*	4.063	1579.700	0.190	0.482	1.173	1660.400	0.120	0.658
PCB-123	-68.790	412.760	-0.160	0.553	-0.495	252.550	-0.062	0.818	-0.318	275.080	-0.088	0.746
PCB-156	-11.972	908.880	-0.028	0.917	1.339	776.590	0.172	0.525	0.867	714.330	0.244	0.362
PCB-157	-83.943	467.990	-0.409	0.116	1.400	125.700	0.369	0.159	0.414	151.630	0.240	0.371
PCB-167	-4.697	288.730	-0.031	0.908	-0.497	311.720	-0.180	0.505	-0.242	320.090	-0.193	0.475
PCB-189	12.291	217.360	0.095	0.727	0.513	214.910	0.215	0.425	0.343	189.020	0.315	0.234
non-ortho PCBs	-63.683	690.300	-0.192	0.476	0.057	504.230	0.009	0.973	0.040	500.940	0.014	0.958
mono-ortho PCBs	-920.020	6925.300	-0.443	0.086	6.304	3835.700	0.165	0.543	2.146	3900.700	0.123	0.650
∑DL-PCBs	-983.700	7615.600	-0.436	0.091	6.361	4340.000	0.153	0.572	2.186	4401.600	0.115	0.671
	Sperm progressive motility (%)				**Sperm viability (%)**				**Normal morphology (%)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	6.370	-156.100	0.262	0.327	11.561	-781.090	0.237	0.377	38.618	-298.840	0.553	0.026*
PCB-81	0.220	44.406	0.125	0.646	1.260	-51.895	0.356	0.177	0.574	49.745	0.113	0.677
PCB-126	-1.256	174.210	-0.210	0.434	-1.563	235.250	-0.131	0.630	-0.035	100.030	-0.002	0.994
PCB-169	0.088	123.630	0.014	0.958	-1.183	231.560	-0.096	0.723	-1.732	152.230	-0.099	0.717
PCB-105	2.992	273.570	0.126	0.641	11.653	-560.270	0.245	0.360	23.559	133.330	0.346	0.189
PCB-114	-0.768	167.500	-0.097	0.722	2.370	-83.855	0.149	0.583	-2.187	151.390	-0.096	0.724
PCB-118	9.405	1318.300	0.113	0.677	54.044	-2814.400	0.323	0.222	42.582	1302.500	0.178	0.509
PCB-123	8.380	-281.780	0.272	0.308	14.170	-1013.800	0.229	0.393	49.420	-450.900	0.559	0.024*
PCB-156	-13.256	1662.600	-0.437	0.090	-10.384	1776.100	-0.171	0.527	-23.033	1185.600	-0.265	0.322
PCB-157	-2.420	372.050	-0.164	0.543	0.709	166.640	0.024	0.930	-2.848	266.670	-0.067	0.804
PCB-167	0.620	238.480	0.058	0.832	3.675	-43.690	0.171	0.527	8.333	162.810	0.270	0.311
PCB-189	-4.839	540.080	-0.521	0.039*	-8.478	988.450	-0.455	0.076	-6.190	336.020	-0.232	0.387
non-ortho PCBs	5.422	186.150	0.228	0.396	10.074	-366.170	0.211	0.433	37.425	3.167	0.548	0.028*
mono-ortho PCBs	0.113	4290.800	0.001	0.998	67.760	-1584.900	0.227	0.398	89.638	3087.400	0.210	0.436
∑DL-PCBs	5.535	4476.900	0.034	0.900	77.834	-1951.000	0.240	0.371	127.060	3090.600	0.274	0.305

β_1 indicate regression slope and β_0 the intercept. Significant correlation *(r* Pearson) at $p<0.05$ (*)

Table 6. Multivariate regression between semen parameters and levels of DL-PCBs for low and normal quality semen participants.

	Semen volume (mL)				**Sperm concentration (x10^6 /mL)**				**Sperm total count (x10^6)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	0.035	348.470	0.000	1.000	-1.887	444.010	-0.216	0.252	-0.843	446.720	-0.223	0.235
PCB-81	2.346	57.574	0.090	0.636	-0.057	66.763	-0.085	0.656	-0.036	68.113	-0.126	0.508
PCB-126	24.824	57.311	0.285	0.126	-0.374	143.150	-0.168	0.375	-0.078	133.380	-0.082	0.668
PCB-169	11.414	146.750	0.081	0.669	-0.761	216.040	-0.213	0.259	-0.418	226.200	-0.270	0.148
PCB-105	30.898	540.610	0.089	0.641	-1.962	723.200	-0.220	0.242	-1.028	743.590	-0.267	0.153
PCB-114	13.952	115.630	0.127	0.505	-0.712	189.270	-0.253	0.177	-0.282	186.070	-0.232	0.217
PCB-118	91.291	2012.900	0.092	0.627	-1.361	2327.900	-0.054	0.777	-1.333	2414.200	-0.122	0.520
PCB-123	10.320	177.250	0.041	0.829	-0.422	226.440	-0.066	0.729	-0.139	221.240	-0.050	0.792
PCB-156	87.002	786.890	0.180	0.340	-1.249	1084.700	-0.101	0.594	-0.762	1110.200	-0.143	0.450
PCB-157	-5.222	259.280	-0.037	0.845	0.682	210.720	0.190	0.315	0.156	227.080	0.100	0.598
PCB-167	49.841	149.880	0.316	0.089	-0.795	324.520	-0.197	0.296	-0.361	326.280	-0.207	0.272
PCB-189	45.535	214.490	0.201	0.286	-0.993	387.540	-0.172	0.363	-0.453	389.980	-0.181	0.337
non-ortho PCBs	38.620	610.100	0.095	0.619	-3.078	869.960	-0.295	0.113	-1.376	874.410	-0.306	0.100
mono-ortho PCBs	323.620	4256.900	0.166	0.379	-6.813	5474.300	-0.137	0.470	-4.202	5618.600	-0.196	0.300
∑DL-PCBs	362.240	4867.000	0.167	0.377	-9.891	6344.200	-0.179	0.345	-5.578	6493.000	-0.233	0.215
	Sperm progressive motility (%)				**Sperm viability (%)**				**Normal morphology (%)**			
PCBs	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***	β_1	β_0	***r***	***p***
PCB-77	-2.718	460.350	-0.167	0.379	-5.434	758.100	-0.243	0.195	16.338	190.080	0.231	0.220
PCB-81	-0.083	67.303	-0.067	0.727	0.103	56.175	0.060	0.752	0.722	56.900	0.134	0.481
PCB-126	-1.583	189.360	-0.381	0.037*	-3.164	362.720	-0.557	0.001*	-3.953	162.600	-0.220	0.244
PCB-169	-2.334	273.530	-0.350	0.058	-4.963	551.590	-0.542	0.002*	-10.365	278.080	-0.357	0.052
PCB-105	-5.256	840.150	-0.317	0.088	-7.227	1168.600	-0.318	0.087	-0.548	629.250	-0.008	0.968
PCB-114	-1.593	218.780	-0.304	0.103	-2.117	312.820	-0.295	0.114	-3.832	190.430	-0.168	0.374
PCB-118	-10.894	2707.200	-0.231	0.219	-19.430	3723.400	-0.301	0.106	-42.745	2673.700	-0.209	0.268
PCB-123	0.795	172.380	0.067	0.727	0.689	153.130	0.042	0.825	19.345	17.432	0.373	0.042*
PCB-156	-7.478	1329.100	-0.325	0.079	-6.479	1509.800	-0.206	0.275	-32.589	1337.600	-0.326	0.078
PCB-157	-0.350	259.610	-0.052	0.784	-1.391	350.030	-0.152	0.424	-1.747	262.150	-0.060	0.753
PCB-167	-1.369	340.610	-0.182	0.336	-0.416	315.650	-0.040	0.832	-1.890	302.610	-0.058	0.762
PCB-189	-4.424	519.260	-0.410	0.024*	-8.575	983.540	-0.580	0.001*	-20.616	537.250	-0.440	0.014*
non-ortho PCBs	-6.717	990.550	-0.346	0.061	-13.459	1728.600	-0.505	0.004*	2.741	687.660	0.032	0.865
mono-ortho PCBs	-30.569	6387.100	-0.330	0.075	-44.946	8517.000	-0.354	0.055	-84.622	5950.400	-0.210	0.265
∑DL-PCBs	-37.285	7377.600	-0.361	0.050	-58.405	10246.000	-0.412	0.023*	-81.880	6638.100	-0.183	0.334

β_1 indicate regression slope and β_0 the intercept. Significant correlation *(r* Pearson) at $p<0.05$ (*)